PHYTOCHEMICAL AND SELECTED PHARMACOLOGICAL STUDIES OF STANDARDIZED FRUIT EXTRACTS OF *MORINDA CITRIFOLIA* LINN.

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By

BEH HOOI KHENG

Thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

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Dedicated to my parents, siblings, nephews and niece

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Finally, my best wishes to all of those who have supported me in any respect during these years.

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ALT	Alanine transaminase
AST	Aspartate aminotransferase
ATCC	American Type Culture Collection
BHA	Butylated hydroxyanisole
BHK	Baby hamster kidney
BHT	Butylated hydroxytoluene
BMI	Body Mass Index
BuOH	Butanol
CAM	Chick Chorioallantoic Membrane
$CHCl_3$	Chloroform
cm	Centimeter
CO_2	Carbon dioxide
DMSO	Dimethyl sulfoxide
DPPH	1,1-Diphenyl-2-picrylhydrazyl
EA	Ethyl acetate
EDTA	Ethylenediaminetetraacetic acid
FCR	Folin-Ciocalteu's reagent
FTC	Ferric thiocyanate
GAE	Gallic acid equivalent
GC-MS	Gas chromatography - mass spectrometry
g	Gram

HCl	Hydrochloric acid
HCT116	Human colon cancer
HDL	High-density lipoprotein
HEp2	Human laryngeal epithiloma
HFD	High fat diet
HL-60	Human promyelocytic leukemia cell lines
HPLC	High-performance liquid chromatography
i.d	Internal diameter
kg	Kilograms
L	Liter
LC_{50}	Median lethal concentrations
LDL	Low-density lipoprotein
Na_2CO_3	Sodium carbonate
m	Meter
MCF-7	Human breast cancer cell lines
mg	Milligram
min	minute
mL	Milliliter
mm	Millimeter
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NaOH	Sodium hydroxide
nm	Nanometer

OD	Optical density
PBS	Phosphate buffer saline
ppm	Part per million
QE	Quercetin equivalent
R_f	Retardation factor
S.D	Standard deviation
TBA	Thiobarbituric acid
TC	Total cholesterol
TG	Triglyceride
TLC	Thin layer chromatography
TWEEN	Polysorbate
U	Unit
UV	Ultraviolet
Vero	African green monkey kidney
WHO	World Health Organization
w/w	Weight over weight
Y79	Retinoblastoma
µg	Microgram
µL	Microliter
µm	Micrometer
^oC	Celsius degree

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KAJIAN FITOKIMIA DAN FARMAKOLOGI TERPILIH UNTUK EKSTRAK TERPIAWAI BUAH *MORINDA CITRIFOLIA* LINN.

ABSTRAK

Morinda citrifolia Linn. biasanya digunakan dalam kalangan orang tempatan Malaysia sebagai ubat tradisional untuk pelbagai jenis penyakit. Kini, terdapat sebelas jenis produk komersial buah *Morinda citrifolia* berdaftar di pasaran Malaysia. Dalam kajian ini, penyaringan fitokimia permulaan dan kajian fizikal-kimia untuk serbuk buah diselidiki dan parameter kawalan mutu yang melibati profil kromatografi ekstrak dijalankan untuk pemiawaian tumbuhan.

Kesan anti-angiogenik dari ekstrak buah telah dinilai dengan menggunakan kaedah membran korioalantoik embrio ayam (CAM). Ekstrak metanol dan fraksi kloroform memberikan kesan anti-angiogenik dengan nilai skor 0.94 dan 1.22 masing-masing. Pengasingan bioaktiviti-bimbingan telah dilakukan dan scopoletin telah dikenalpastikan sebagai salah satu sebatian aktif dalam aktiviti anti-angiogenik (nilai skor 1.39).

Kesan sitotoksik ekstrak buah telah diperhati pada sel kanser payudara (MCF-7) dan sel leukemia manusia (HL-60) dengan menggunakan assai MTT. Ekstrak metanol telah merencat 37.6% sel MCF-7 dan 25.62% sel HL-60 pada kepekatan

30 ug/mL. Fraksi-fraksi yang lain dari buah talah menunjukkan aktiviti sitotoksik yang lebih lemah berbanding dengan ekstrak metanol.

Adipogenesis disertai oleh pertumbuhan salur darah yang baru, dan dengan itu, penindasan angiogenesis akan menghalang adipogenesis dan obesiti. Kesan anti-obesiti dan anti-hiperlipidemia telah diuji ke atas ekstrak metanol dan fraksi kloroform dengan menggunakan tikus hiperlipidemia induksi diet tinggi lemak. Hasilnya menunjukkan ekstrak buah mempengaruhi metabolisme trigliserida dan kolesterol pada tikus obesiti. Ekstrak buah juga mengurangkan jumlah peningkatan berat badan tikus dengan ketara.

Kajian antioksidan ekstrak buah telah dinilai dalam kajian ini. Fraksi etil asetat mengandungi kandungan fenolik (167.71 ± 5.30 ug/mL GAE) dan kandungan flavonoid (22.30 ± 1.22 ug/mL QE) yang tertinggi. Fraksi tersebut juga mempunyai aktiviti antioksidan yang tertinggi dalam penangkapan radikal DPPH dengan nilai EC_{50} 164.09 ug/mL and mempunyai 83.46% aktiviti antioksidan dalam ujian *β*-karoten-linoleat pada kepekatan 500 ug/mL.

Kajian ini membekalkan maklumat kawalan mutu dan pemiawaian untuk ekstrak buah *Morinda citrifolia*. Kajian anti-angiogenik, sitotoksisiti, anti-obesiti, anti-hiperlipidemia dan antioksidan membuktikan kegunaan tradisional buah ini untuk penyakit yang berkaitan.

PHYTOCHEMICAL AND SELECTED PHARMACOLOGICAL STUDIES OF STANDARDIZED FRUIT EXTRACTS OF *MORINDA CITRIFOLIA* LINN.

ABSTRACT

Morinda citrifolia is commonly used in Malaysia by locals as traditional medicine for various diseases. Currently there are eleven registered commercial products of *Morinda citrifolia* fruit available in Malaysia. In this study, preliminary phytochemical screening and physico-chemical studies of the fruit powder were attempted and quality parameter involving chromatographic profiling of the extracts were carried out for standardization.

The fruit extracts were screened for anti-angiogenic effect using *in vivo* chick chorioallantoic membrane assay. Methanolic extract and chloroform fraction showed anti-angiogenic effect with the score value of 0.94 and 1.22, respectively. Bioactivity-guided isolation was performed and scopoletin was identified as one of the active constituents in anti-angiogenic activity (score value 1.39).

Cytotoxic effects of the fruit extracts were observed on breast cancer cell lines (MCF 7) and human leukemia cell lines (HL-60) using MTT cell viability assay. The methanolic extract inhibited 37.6% of MCF 7 cells and 25.62% of HL-60 cells

at concentration of 30 ug/mL. The other fractions of the fruit showed weaker cytotoxic activity than methanolic extract.

Adipogenesis is concomitantly accompanied by new blood vessel growth, and thus suppression of angiogenesis would prevent adipogenesis and obesity. Methanolic extract and chloroform fraction were tested for their anti-obesity and anti-hyperlipidemic effects using high fat diet induced hyperlipidemic rats. The finding showed the fruit extracts influenced triglyceride and cholesterol metabolism in obese rats. The fruit extracts also significantly reduced the percentage of total body weight increased in the rats.

Antioxidant activity of fruit extracts was evaluated in this study. Ethyl acetate fraction contains the highest phenolic content (167.71 ± 5.30 ug/mL GAE) and flavonoid content (22.30 ± 1.22 ug/mL QE). The fraction also possesses the highest antioxidant activity in DPPH scavenging with EC_{50} value 164.09 ug/mL and 83.46% antioxidant activity in β-carotene-linoleate assay at the concentration of 500 ug/mL.

The present studies provided information on the quality and standardization of the fruit extracts. The anti-angiogenic, cytotoxicity, anti-obesity, anti-hyperlipidemic and antioxidant studies of the fruit provided evidence on the traditional use for related disease.

CHAPTER 1

INTRODUCTION

1.1 General

Herbal medicines are the therapeutic experiences of generations of practicing physicians of traditional medicine over hundreds of years and they are known to be oldest health care products that have been used by mankind all over the world to treat various types of ailments (Torey *et al*., 2010). Recently, considerable attention has been paid to utilize eco-friendly and bio-friendly plant-based products for the prevention and cure of different human diseases. It is documented that 80% of the world's population has faith in traditional medicine, particularly plant drugs for their primary healthcare (Dubey *et al*., 2004).

Modern technological medicine is nowadays much criticized for waiting for diseases to occur and then trying to cure it rather than seeking to prevent it from occurring in the first place (Laurence & Black, 1978). Herbal products have played an important role today not only to heal the diseases but also to prevent the diseases from occurring. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body and lesser side effects (Kamboj, 2000).

There are estimated 350,000 flowering plant species identified so far, about 35,000 species are used worldwide for medicinal purposes (Kong *et al*., 2003). However,

the number could be much higher as knowledge on the indigenous uses of plants was mostly passed on orally from one generation to another and has largely remained undocumented (Jantan, 2004). Tropical rainforests cover about 12 % of the land area of the earth, Kong *et al*. (2003) reported that tropical rainforests are a vital source of medicines, there are not more than 1 % of the world's tropical forest plants have been tested for pharmaceutical properties, yet at least 25 % of all modern drugs originally came from rainforests.

Morinda citrifolia is commonly used in Malaysia by local people as folk remedy to cure or to prevent diseases. There are eleven registered commercial products of *M. citrifolia* fruit available in the market. In Malaysia, *Morinda citrifolia* L. (mengkudu besar) and *Morinda elliptica* Ridl. (mengkudu hutan) are the two common *Morinda* species. Both of the species have their therapeutic effect. *M. citrifolia* is widely used to treat diabetes and *M. elliptica* is widely used to treat diarrhoea (Ong & Nordiana, 1999).

1.2 Phytochemical and biological activities of genus *Morinda*

The genus *Morinda* (Rubiaceae) is made up of around 80 species (Chan-Blanco *et a*l., 2006). In the Indo-Pacific region, species diversity is highest in Near Oceania with attenuation into Remote Oceania. The genus *Morinda* includes trees, shrubs, and vines (McClatchey, 2003). *Morinda* is a genus of the family Rubiaceae and has long been known to contain substantial amount of anthraquinones. About 90%

of these compounds occur as derivatives of 9,10-anthracenedione with several hydroxy and other functional groups, such as methyl, hydroxymethyl and carboxyl (Jasril *et al*., 2003). Hydroxyanthraquinones are the active principles of many phyto-therapeutic drugs (Wolfle *et al*., 1990).

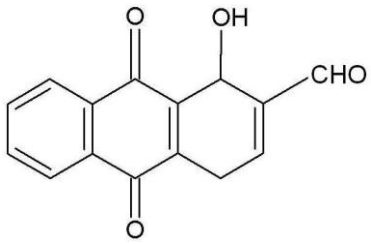
1.2.1 *Morinda elliptica*

Morinda elliptica Ridl. is a small plant known as "mengkudu kecil". It is a shrub or small tree, growing wild in newly developed arcas or in bushes. It is a native plant of Asia and Polynesia used in traditional folk medicine such as cholera, diarrhea, piles, headache and to increase appetite (Ismail *et al*., 1997; Ishak *et al*., 2010).

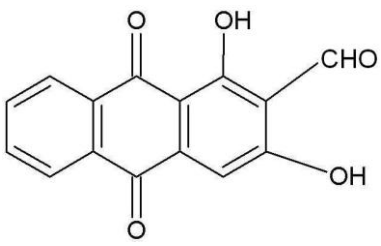
Ismail *et al*. (1997) reported a new anthraquinone and 10 known anthraquinones. The anthraquinone, 2-formyl-1-hydroxyanthraquinone and 10 known anthraquinones, 1-hydroxy-2-methylanthraquinone, nordamnacanthal, damnacanthal, lucidin-ω-methyl ether, rubiadin, soranjidiol, morindone, rubiadin-l-methyl ether, alizarin-l-methyl ether and morindone-5-methyl ether were isolated from roots of *M. elliptica*. The structures of the isolated compounds were shown in Figure 1.1.

Jasril *et al*. (2003) tested the antitumor and antioxidant activities of six anthraquinones (nordamnacanthal, alizarin-1-methyl ether, rubiadin, soranjidiol,

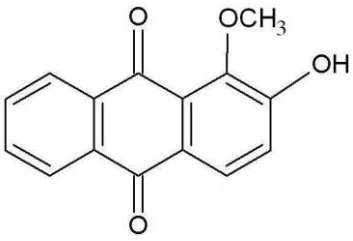
lucidin-ω-methyl ether and morindone) of *M. elliptica*. All compounds exhibited stronger antitumor activity than the reference compounds genistein and quercetin. In antioxidant assay using ferric thiocyanate (FTC) method, nordamnacanthal and morindone showed stronger antioxidant activity than α-tocopherol. However when the compounds were assayed for scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals, only morindone was considered to be active as free radical scavenger. This observation suggested that radical scavenging is less prominent in nordamnacanthal as compared to morindone. The differences between the two compounds are that the formyl at C-2 and hydroxyl at C-3 in nordamnacanthal are replaced by a methyl and proton groups, respectively in morindone.



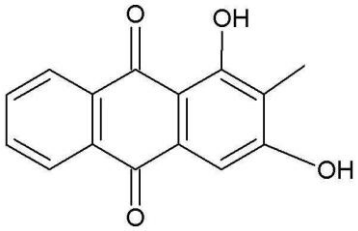
1. 2-formyl-1-hydroxyanthraquinone



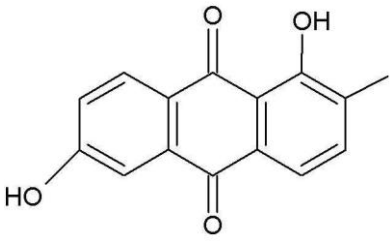
2. nordamnacanthal



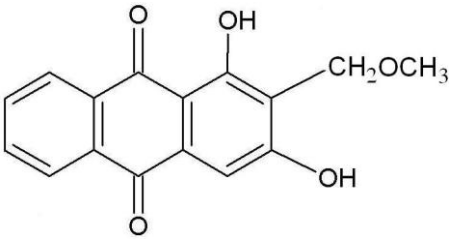
3. alizarin-1-methyl ether



4. rubiadin

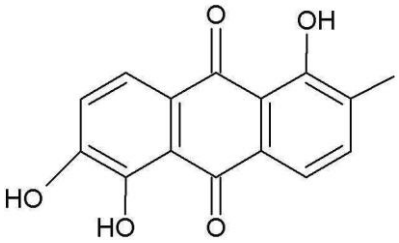


5. soranjidiol

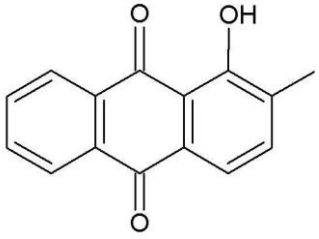


6. lucidin-ω-methyl ether

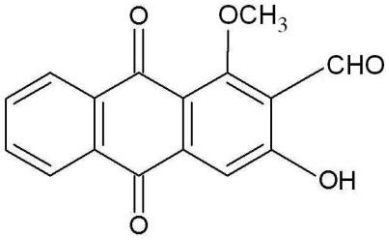
Figure 1.1 Isolated compounds from the roots of *Morinda elliptica*



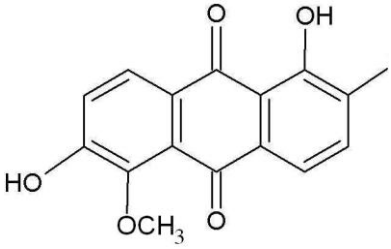
7. morindone



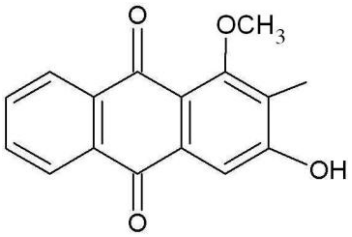
8. 1-hydroxy-2-methylanthraquinone



9. damnacanthal



10. morindone-5-methyl ether



11. rubiadin-l-methyl ether

Figure 1.1 (continued) Isolated compounds from the roots of *Morinda elliptica*

1.2.2 *Morinda morindoides*

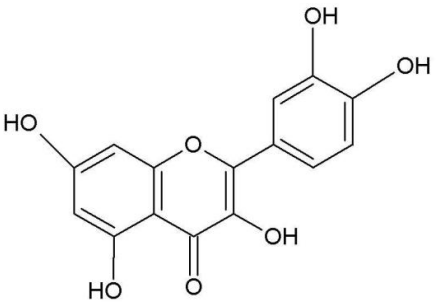
Morinda morindoides Milne-Redh, is one of the most popular medicinal plants currently used in villages and towns in Democratic Republic of Congo in traditional medicine. An aqueous decoction of fresh leaves, which is the typical traditional remedy used for the treatment of various illnesses among which are diarrhoea and constipation associated with intestinal worms (Cimanga *et al*., 2010).

Marie-Genevieve *et al*. (2010) reported that the *in vitro* effect of the toluene, methyl tert-butyl ether (MtBE), ethyl acetate (EtOAc), n-butanol (n-BuOH) and water extracts from *M. morindoides* leaves on their cytotoxicity effect against leukemic cell lines. They found that both toluene and MtBE extracts exhibited a significant cytotoxic effect on the cell lines. The highest cytotoxicity was obtained with toluene extract. By contrast, EtOAc, n-BuOH and water extracts did not affect cell viability of the three cell lines tested.

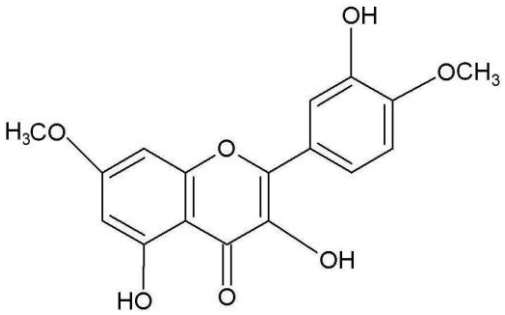
Cimanga and co-workers reported *M. morindoides* leaf extracts have been shown to possess antiprotozoal activity particularly against *Entamoeba histolytica* (Cimanga *et al*., 2006). Extracts, fractions and some isolated compounds from *M. morindoides* leaves were tested for their potential *in vitro* antiamoebic activity. Results indicated that the aqueous decoction (dried extract) and 80% methanolic extract displayed an appreciable antiamoebic activity. The $CHCl_3$, EtOAc and *n*-BuOH soluble fractions from the partition of 80% methanolic extract exhibited an

average antiamoebic activity. The residual water-soluble fraction showed a weak effect against *Entamoeba histolytica*.

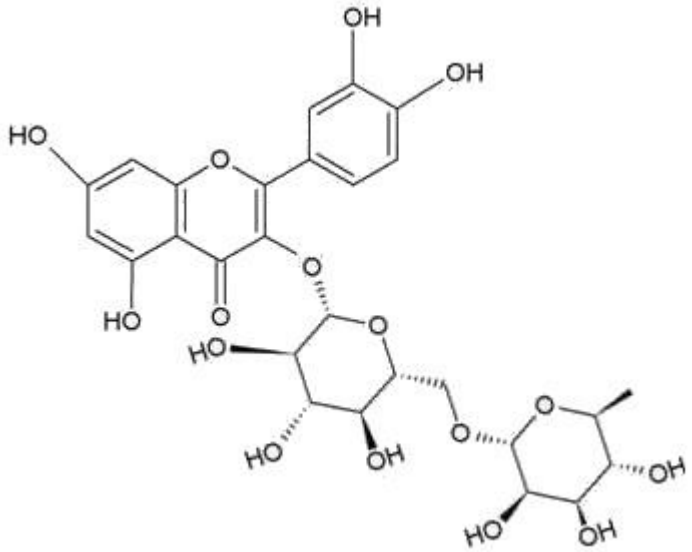
A number of isolated compounds from the leaf of *M. morindoides* were reported (Cimanga *et al*., 2006). The structures of the compounds are shown in Figure 1.2.



12. quercetin

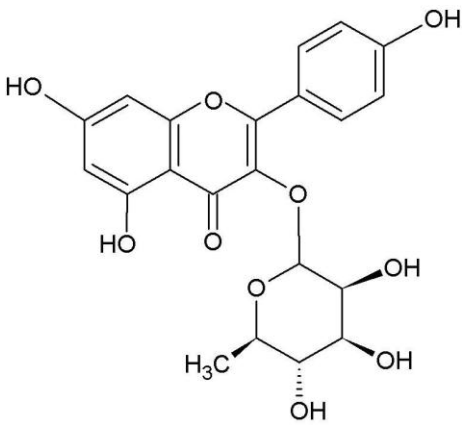


13. quercetin-7,4 prim-dimethylether

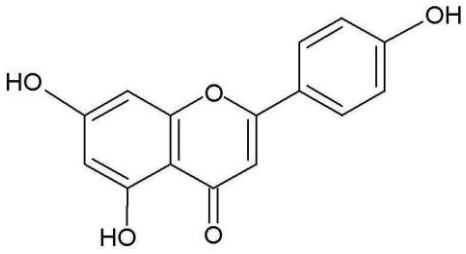


14. quercetin-3-O-rutinoside

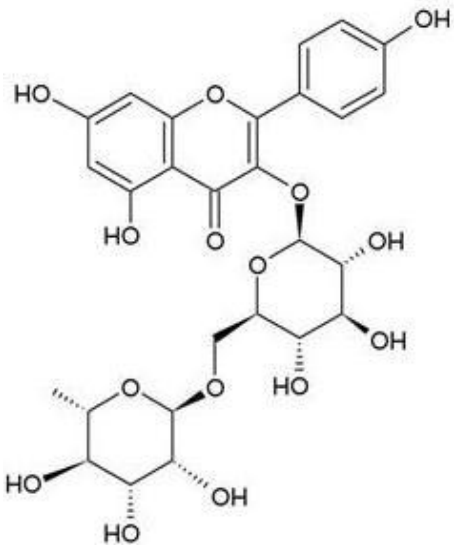
Figure 1.2 Isolated compounds from the leaf of *Morinda morindoides*



15. kaempferol-3-O-rhamnoside

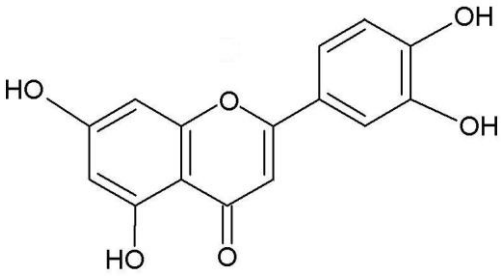


16. kaempferol

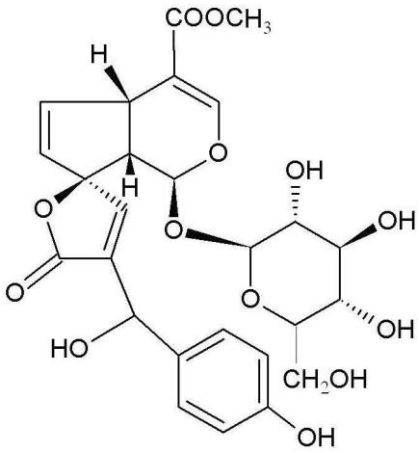


17. kaempferol-3-O-rutinoside

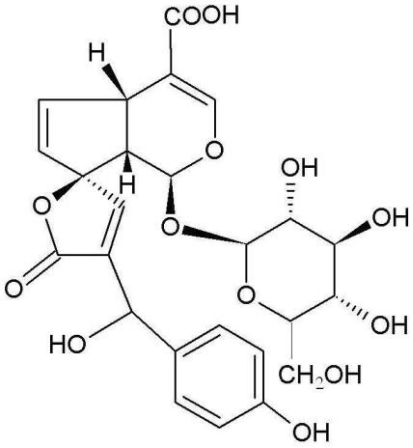
Figure 1.2 (continued) Isolated compounds from the leaf of *Morinda morindoides*



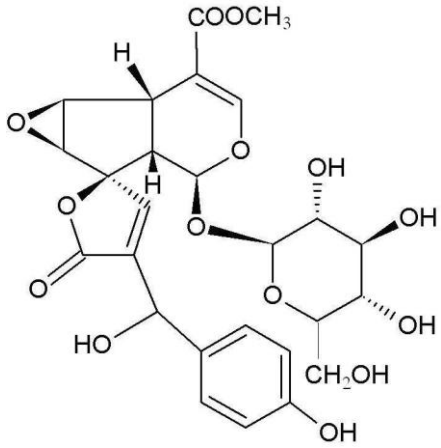
18. luteolin



19. gaertneroside



20. gaertneric acid



21. epoxygaertneroside

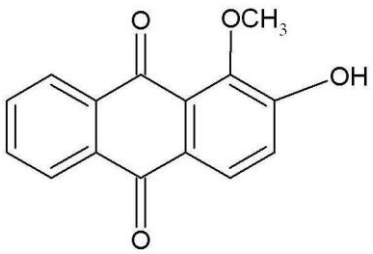
Figure 1.2 (continued) Isolated compounds from the leaf of *Morinda morindoides*

1.2.3 *Morinda officinalis*

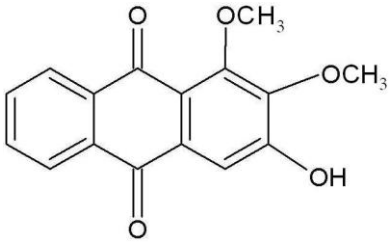
Morinda officinalis How, is one of the traditional Chinese plants grows in humid areas of southeast China. It has been reported to possess the ability to reinforce kidney function, strengthen the tendons and bones and relieve rheumatic condition. This plant is also claimed for its anti-diabetic effects and may have an antidepressant-like action (Soon & Tan, 2002; Zhang *et al*., 2002).

Zhang and co-workers (2009) reported the polysaccharides from the roots of *M. officinalis* were found to have significant anti-fatigue activity by using mice weight-loaded swimming model. The activity may be related to the anti-stress and enhancing immunity effects of *M. officinalis*. The anti-fatigue activity of the polysaccharides may partially explained the tonic property in traditional medicine, which provided scientific evidence for traditional medicine and further development of medicinal products for prevention and treatment of diseases related to chronic fatigue syndromes.

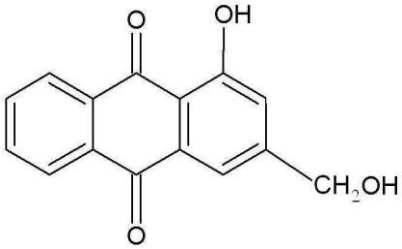
Five anthraquinones including alizarin-1-methylether, 1,2-dimethoxy-3 hydroxyanthraquinone, 1-hydroxy-3-hydroxymethylanthraquinone, rubiadin-1-methylether and anthragallol-2-methylether were isolated from the dried roots of *M. officinalis* How (Zhu *et al*., 2009). The structures of the isolated compounds are shown in Figure 1.3.



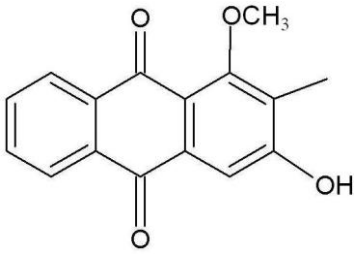
22. alizarin-1-methylether



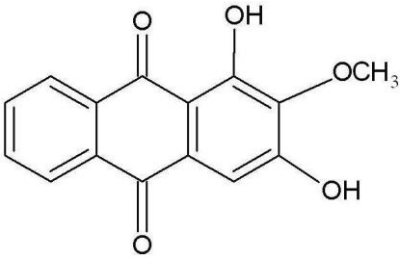
23. 1,2-dimethoxy-3 hydroxyanthraquinone



24. 1- hydroxy-3-hydroxymethylanthraquinone



25. rubiadin-1-methylether



26. anthragallol-2-methylether

Figure 1.3 Compounds isolated from the dried roots of *M. officinalis* How

1.2.4 *Morinda lucida*

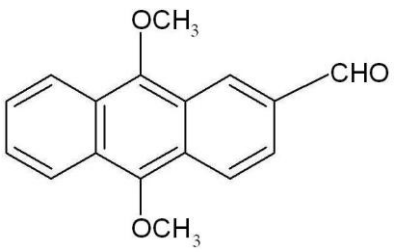
Morinda lucida Benth, is a tropical West Africa rainforest tree also called Brimstone tree. Different parts of the plant are attributed with diverse therapeutic benefits. For example, in Southern Cameroon, cold decoction of the plant leaves is used for the treatment of fever. However, in most parts of West Africa, the bitter water decoction of the plant bark, root and leaf are used as bitter tonic and as astringent for dysentery, abdominal colic and intestinal worm infestation (Adeneye & Agbaje, 2008).

Raji *et al*. (2005) investigated the effect of *M. lucida* on the reproductive activity of male albino rats. *M. lucida* leaf extract did not cause any changes in body and somatic organ weights, but significantly increased the testis weight ($P < 0.05$). The sperm motility and viability, and the epididymal sperm counts of rats treated for 13 weeks were significantly reduced ($P < 0.05$). Sperm morphological abnormalities and serum testosterone levels were significantly increased ($P < 0.05$). There were various degrees of damage to the seminiferous tubules. The extract reduced the fertility of the treated rats by reducing the litter size.

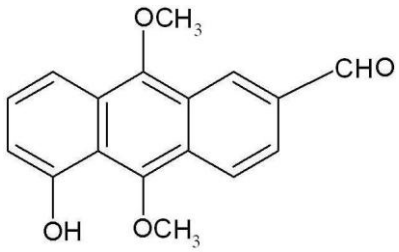
The antibacterial activity of *M. lucida* was investigated against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhi* using the Kirby–Bauer agar diffusion method. The bark methanolic extract of *M. lucida* inhibited the growth of the above mentioned

bacteria with the MIC values below 20 mg/mL. Phytochemical screening was performed on the plant extract and the tests suggested the presence of saponins, flavonoids, alkaloids, terpenoids, tannins and anthraquinones in the plant (Gbedema *et al*., 2010).

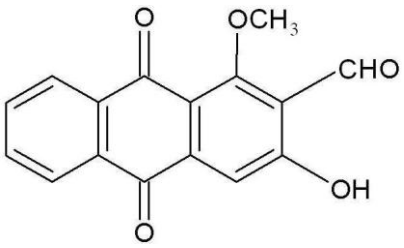
The chemical constituents isolated from *M. lucida* were reported by Adesogan (1973). The structures of these chemical constituents are shown in Figure 1.4.



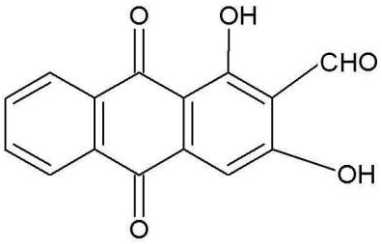
27. oruwal



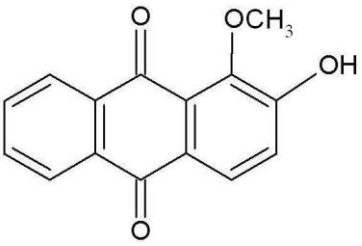
28. oruwalol



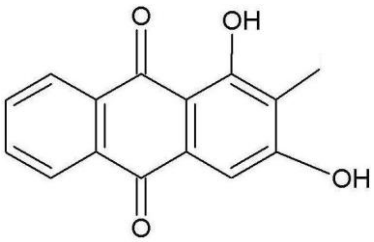
29. damnacanthal



30. nordamnacanthal

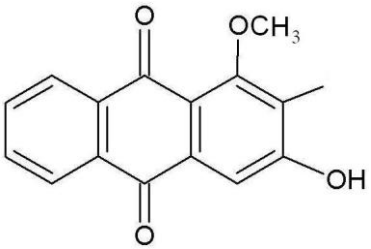


31. alizarin-1-methyl ether

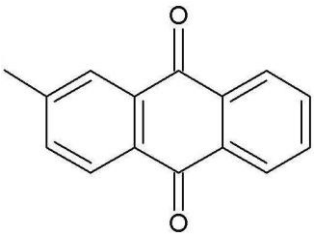


32. rubiadin

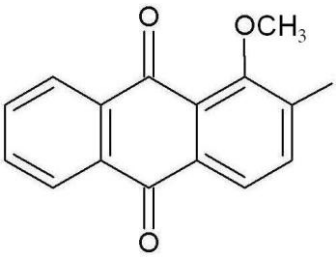
Figure 1.4 Compounds isolated from *Morinda lucida* Benth.



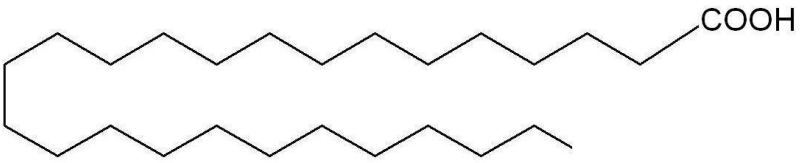
33. rubiadin-1-methylether



34. 2-methyl-anthraquinone



35. 1-methoxy-2-methyl-anthraquinone



36. hexacosanoic acid

Figure 1.4 (continued) Compounds isolated from *Morinda lucida* Benth.

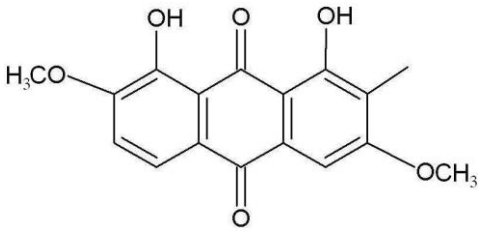
1.2.5 *Morinda angustifolia*

Morinda angustifolia Roxb, is a resourceful perennial undershrub, and widely distributed in the southwestern mountainous areas of China, and nearby countries, such as Burma, Laos, Thailand and India. The major usage of the plant is to make yellow fabric dye stuff (Xiang *et al*., 2008).

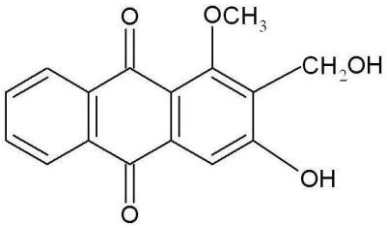
Bhuyan and Saikia (2005) reported that the isolated morindone from the root benzene extract of *M. angustifolia* was the colour component responsible for dyeing. Dyes derived from natural sources have emerged as important alternatives to synthetic dyes, which have been reported to have carcinogenic effects. Recently with the worldwide concern over the use of eco-friendly and biodegradable materials, the use of natural dyes has once again gained interest.

Xiang and co-workers (2008) studied the antimicrobial activity of *M. angustifolia*. Six isolated compounds (1,8-dihydroxy-2-methyl-3,7- dimethoxyanthraquinone, lucidin 3-O-β-primeveroside, 1,3-dihydroxy-2-methylanthraquinone, lucidin- ω - ethyl ether, lucidin-ω-butyl ether and damnacanthol) were tested for their antimicrobial potential against *Bacillus subtilis, Escherichia coli, Micrococcus luteus, Sarcina lutea, Staphylococcus aureus, Aspergillus niger, Candida albicans* and *Saccharomyces sake*. Among the compounds, 1,8-dihydroxy-2-methyl-3,7-dimethoxy anthraquinone demonstrated the most significant antimicrobial activity against *Bacillus subtilis, Escherichia coli, Micrococcus luteus, Sarcina lutea,*

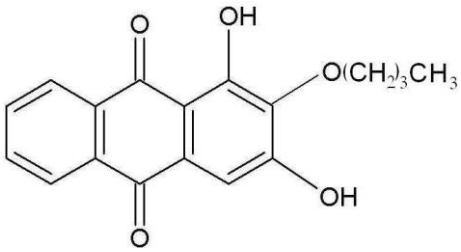
Candida albicans and *Saccharomyces sake*. The structures of the isolated compounds are shown in Figure 1.5.



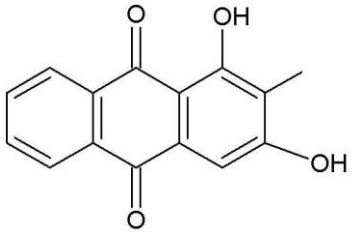
37. 1,8-dihydroxy-2-methyl-3,7-dimethoxyanthraquinone



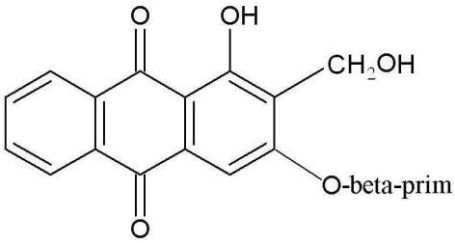
38.damnacanthol



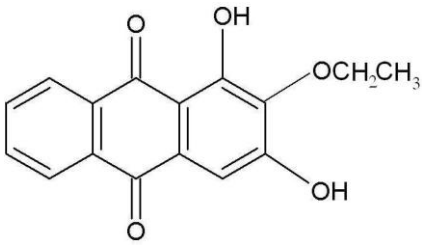
39. lucidin-ω-butyl ether



40. 1,3-dihydroxy-2-methylanthraquinone



41. lucidin 3-O-β-primeveroside



42. lucidin- ω -ethyl ether

Figure 1.5 Structures of the isolated compounds from the roots of *M. angustifolia*

1.3 Plant *Morinda citrifolia* Linn.

1.3.1 Plant taxonomy

Kingdom: Plantae

Sub kingdom: Tracheobionta

Super division: Spermatophyta

Division: Magnoliopsida

Class: Magnoliopsidae

Subclass: Asteridae

Order: Rubiales

Family: Rubiaceace

Genus: *Morinda*

Species: *Morinda citrifolia*

Common name: Noni (Hawaii or island of Polinesia) or mengkudu (Malaysia)

Retrieved from: http://ethnoakmal.blogspot.com/2009/02/mengkudu-noni-morinda-citrifolialinn.html (September, 2011)

Figure 1.6 The fruit of *Morinda citrifolia* (with flowers)

Figure 1.7 Young fruit of *Morinfa citrifolia*

1.3.2 Plant morphology

Morinda citrifolia is a bush or small tree, 3–10 m tall, with abundant wide elliptical leaves (5–17 cm length, 10–40 cm width). The small tubular white flowers are grouped together and inserted on the peduncle. The petioles leave ring-like marks on the stalks and the corolla is greenish white (Chan-Blanco *et al*., 2006).

M. citrifolia fruit (Figure 1.6 and Figure 1.7) is oval in shape and will turn from a greenish to a yellowish-white color when it ripens. It has a bitter taste and exhales a strong butyric acid-like pungent smell. The seeds have a distinct air chamber, and can retain viability even after floating in water for months (Nelson, 2003). The roots and inner bark may have little coloration or may range from bright yellow to red.

1.3.3 Plant habitat and distribution

The plant habitat ranges from tropical rainforest to dry lowland plains, from the coast to elevated inland sites. It can tolerate exposed sites as well as the relatively thin and infertile soils. The plant is present worldwide predominantly in tropical countries. *M. citrifolia* occurs in Africa, Australia, Barbados, Cambodia, Caribbean, Cayman Islands, Cuba, Dominican Republic, El Salvador, Fiji, Florida, French West Indies, Guadeloupe, Guam, Haiti, Hawaii, India, Jamaica, Java, Laos, Malaysia, Marquesas Islands, Philippines, Polynesia, Puerto Rico, Raratonga,

Samoa, Seychelles, Solomon Islands, Southeast Asia, St. Croix, Surinam, Tahiti, Thailand, Tonga, Trinida and Tobago and Vietnam (Mathivanan *et al*., 2005).

1.3.4 Uses in traditional medicine

M. citrifolia has been used in folk remedies by Polynesians for over 2000 years, and is reported to have a broad range of therapeutic effects, including antibacterial, antiviral, antifungal, antitumor, analgesic, hypotensive, anti-inflammatory, antidiabetic and immune enhancing effects (Wang *et al*., 2002).

Dixon *et al*. (1999) and Morton (1992) reported the fruit of *M. citrifolia* was traditionally used to treat gum disorder, tuberculosis, anthelmintic and as purgative. The fruit pulp can be used as an insecticidal shampoo. The flower was applied to treat sore eye. The leaves of the plant was used to treat wounds, ulcers, ringworm, rheumatic pain, inflammation, liver diseases, internal bleeding, abdominal swollen, fever, headache, cough and cold. The bark was used to treat malaria and finally the root was consumed to cure hypertension.

Nelson (2003) reported the teas from the leaves were used as treatment for malaria and analgesic in Africa. All parts of the plants are useful laxative. Decoctions of the stem bark are consumed to treat jaundice and the extract of the leaves is for hypertension. Other usages such as to treat sprains, deep bruising, toothaches, fractures, diabetes, loss of appetite, urinary tract ailments, abdominal swelling,

hernias, stings from stonefish and human vitamin A deficiency were also reported by the author.

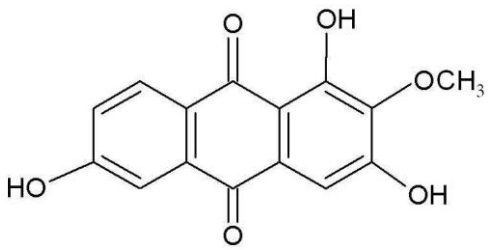
1.3.5 Literature survey

Yong *et al*. (2006) studied the free-radical-scavenging activity of *M. citrifolia* juice and powder in processing and storage. The authors proposed the fresh juice *M. citrifolia* possessed free-radical-scavenging activity (RSA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), at 140 mg equivalent ascorbic acid/100 mL and total phenols at 210 mg gallic acid/100 mL. Fermentation of the fruit for 3 months resulted in a loss of more than 90% of RSA. Dehydration at 50 ^{o}C produced a loss of 20% of RSA. Storage of fresh fruit juice at 24^{o}C for 3 months reduced RSA more than 90%. Storage of fruit juice or powder at -18^{o}C and 4^{o}C for 3 months decreased RSA by 10–55%. For maintenance of the substantial antioxidant properties of *M. citrifolia* fruit products, processing of fruit powder or fresh frozen fruit juice rather than fermented fruit juice is recommended.

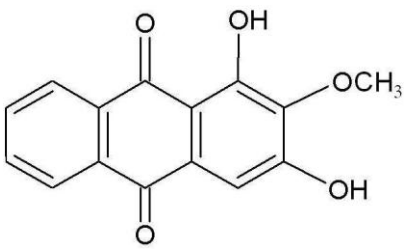
Pawlus *et al*. (2005) isolated a new anthraquinone, 2-methoxy-1,3,6-trihydroxyanthraquinone from the fruit of *M. citrifolia*. The compound was reported to have potent quinone reductase (QR) induction activity, which is 40 times more potent than a positive control, L-sulforaphane. Furthermore, this compound demonstrated no discernible cytotoxicity at the highest dose tested. QR is a phase II metabolizing enzyme. The induction of phase II enzymes is

considered cancer chemopreventive in that potential oxidative and electrophilic molecules can be more readily metabolized and excreted before they can interact with cellular macromolecules such as DNA. QR is also responsible for maintaining the reduced states of antioxidants such as R-tocopherol and coenzyme Q10. Hence, QR inducers are sometimes referred to as "indirect antioxidants", and this activity is considered protective at the initiation stage of carcinogenesis. The chemical structures of 2-methoxy-1,3,6-trihydroxyanthraquinone together with a few known isolated compounds were shown in Figure 1.8.

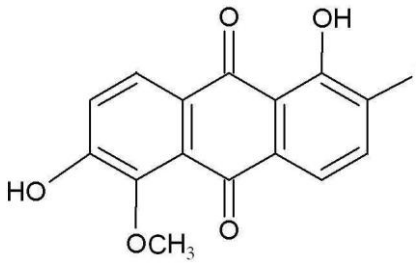
Taskin *et al*. (2009) investigated the apoptosis-inducing effects of *M. citrifolia* and doxorubicin on the Ehrlich ascites tumor in Balb-c mice and also combined it with a potent anti-cancer agent, doxorubicin. The first group of animal received *M. citrifolia* fruit only, the second group of animal received doxorubicin, and the third group of animal received both *M. citrifolia* fruit and doxorubicin for 14 days after the inoculation of cells. The control group received 0.9% NaCl only. The result found that short and long diameters of the tumor tissues were about 40–50% smaller and the proliferation was decreased, compared to those in control group. This anti-growth effect resulted from the induction of apoptosis. The authors concluded *M. citrifolia* fruit may be useful in the treatment of breast cancer either on its own or in combination with doxorubicin.



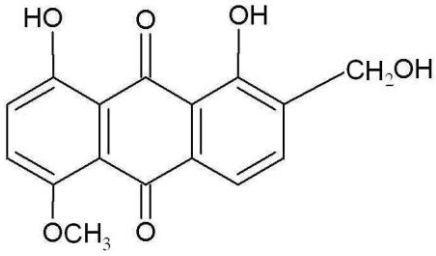
43. 2-methoxy-1,3,6-trihydroxy-anthraquinone



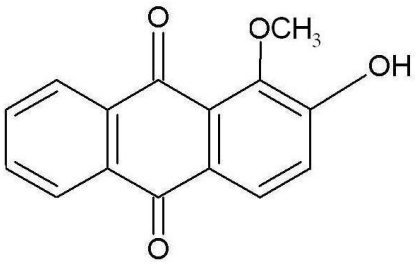
44. 1,3-dihydroxy-2-methoxy-anthraquinone



45. 1,6-dihydroxy-5-methoxy-2-methylanthraquinone



46. 1,8-dihydroxy-2- hydroxymethyl-5-methoxyanthraquinone



47. 2-hydroxy-1-methoxyanthraquinone

Figure 1.8 Isolated compounds by Pawlus *et al*. (2005)